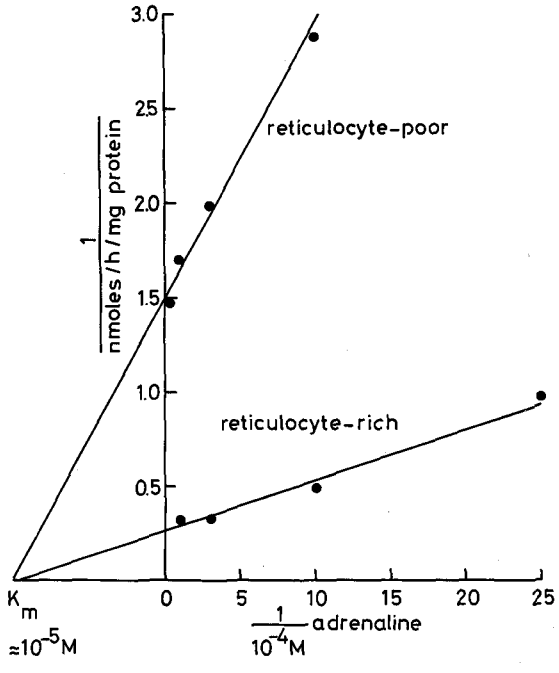
RESCH et al.[14]; reticulocytes were counted in blood smears stained with brilliant cresyl blue.

Results. The results obtained are compiled in the Table. When COMT activities were determined in the 15,000 × *g* supernates of the haemolysates, a slight but insignificant increase of enzyme activity was seen in reticulocyte-rich relative to reticulocyte-poor preparations. In ghost preparations, however, marked differences were observed: COMT activities in membrane fractions from reticulocyterich blood were about 5 times higher than those found in reticulocyte-poor preparations. From these results it is obvious that in contrast to the soluble enzyme, membrane-bound COMT is preferentially localized in the reticulocytes.

As can be seen from the Figure, despite the 5-fold difference in the maximum reaction velocities of COMT activities between reticulocyte-poor and reticulocyte-rich ghost preparations, the apparent K_m values for the substrate adrenaline were identical. This indicates that the COMT activities determined in reticulocyte-poor and reticulocyte-rich ghost preparations come from identical enzymes.

Discussion. Our investigations have revealed that COMT activities in cytoplasmic membranes and cytosol of rat erythrocytes come from two enzymes which are different not only with respect to the criteria previously established[4,5] but also regarding their relative activities in the course of red cell maturation. While soluble COMT activity seems to be evenly distributed throughout the whole erythrocyte population, membrane-bound COMT obviously decreases considerably during the process of erythrocyte maturation. Similar phenomena have been reported for a variety of other enzymes present in red cells[15–18] and also for the adrenergic β-receptor-effector system[8]; the components of the latter (β-sympathomimetically stimulated adenyl cyclase activity, cyclic AMP-phosphodiesterase and cyclic AMP-dependent protein kinase activities) have been shown to be located, at least preferentially, in the reticulocytes. Moreover, recent studies have revealed that considerable monoamine oxidase activity is present in reticulocyte mitochondria[19]. From these results and from those described above, it may tentatively be concluded that, besides binding to blood plasma proteins[20], soluble COMT in the erythrocytes provides an inactivation mechanism for circulating catecholamines, whereas membrane-bound COMT and also monoamine oxidase activities in red cells may be considered to be functionally associated with the adrenergic β-receptor-effector system of the reticulocytes.



Dependence of catechol-*O*-methyltransferase activities in ghost preparations from reticulocyte-poor and reticulocyte-rich erythrocyte suspensions from rats on the substrate (^{3}H-adrenaline) concentrations (Lineweaver-Burk plot). Note that despite a 6-fold higher maximum reaction velocity in reticulocyte-rich preparations, identical apparent K_m values are obtained.

Zusammenfassung. Membranpräparate aus retikulozytenreichem Rattenblut, erzeugt durch Behandlung der Tiere mit Acetyl-Phenylhydrazin, zeigen eine 5–6mal höhere Catechol-*O*-Methyltransferaseaktivität als retikulozytenarme Präparate aus dem Blut unbehandelter Kontrolltiere. Die Aktivität der zytoplasmatischen COMT in den beiden Erythrozytensuspensionen unterscheiden sich nicht signifikant. Offenbar kommt es im Verlauf der Erythrozytenreifung zu einer Abnahme der membranständigen, nicht jedoch der löslichen COMT-Aktivität der roten Blutzellen.

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The Effect of Skim Milk on Plasma Cholesterol in Rats

In experiments dealing with the control of cholesterolemia, we observed that skim milk lowered the levels of plasma cholesterol in rats.

Four timed-pregnant female Sprague-Dawley rats were kept on Purina® rat chow and water. 15 days after delivery, skim milk replaced water on a strictly random basis in 2 of the cages housing the mothers and their offspring. The litters were weaned at 21 days, separated according to sex, and continued either on skim milk or tap water as before. In the litters on skim milk, skim milk was added to the ground chow (1 ml/0.7 g); in the other litters, the chow was mixed with water. At 43 and

Plasma cholesterol concentration in rats on two dietary regimens (mean ± SE)

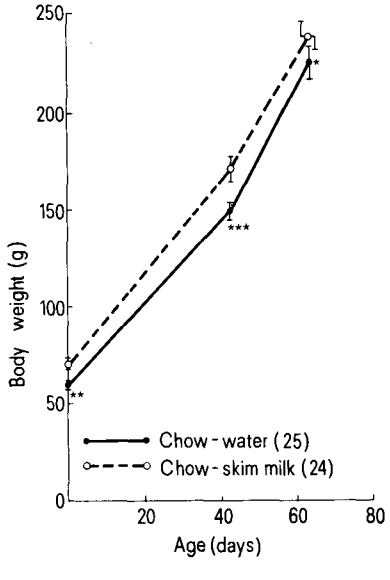
Animals	Plasma cholesterol (mg/100 ml)			
	A) Chow-water		B) Chow-skim milk	
	43-day-old	64-day-old	43-day-old	64-day-old
Males	95 ± 3 (10)	107 ± 3 (10)	76 ± 38[c] (13)	85 ± 4[c] (13)
Females	94 ± 3 (15)	109 ± 2 (15)	109 ± 3[b] (11)	96 ± 4[b] (11)
All rats	95 ± 2 (25)	109 ± 2 (25)	91 ± 4[a] (24)	90 ± 3[c] (24)

p (Student's t-test) A vs B. [a]Nonsignificant; [b]<0.01; [c]< 0.001. Number of rats between parentheses.

64 days of age, the rats were lightly anesthetized with ether, and blood was obtained by nipping the tail. Plasma cholesterol was determined by the $FeCl_3$ method modified by RUDEL and MORRIS[1].

The results (Table) indicate that the male progeny that had received skim milk had lower plasma cholesterol concentrations than those on tap water. These differences were not observed in the female progeny at 43 days, but they were present at 64 days. The rats on the skim milk regimen were initially heavier than those on water, but the weight differences disappeared at 64 days of age (Figure).

We have not investigated the mechanism of this hypocholesterolemic effect of skim milk. According to previous data, the cholesterol concentration in the dam's milk may effect a hypercholesterolemic response in her offspring, but the results reported for rats[2] and rabbits[3] are contradictory. But skim milk given for 6 days before weaning when suckling pups have probably begun to ingest solid food[4] would hardly affect the offspring by possibly changing the cholesterol content of the dam's milk. Moreover, the effect of dam's milk was observed either in the sucklings[5–7] or in weaned animals given exogenous cholesterol[2,3]. None of these conditions were present in our rats.



Body weight changes of rats on two dietary regimens. Between parentheses, number of rats; bars, SE; p (Student's t-test). *Nonsignificant; ** < 0.01; *** < 0.001.

Since the rat chow in our studies contained only a minimal amount of cholesterol (< 0.07 mg/g, unpublished observations), plasma cholesterol was probably mainly biosynthetic in origin. The major regulator of cholesterol biosynthesis is the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A to mevalonic acid[8,9] which is catalyzed by 3-hydroxy-3-methylglutaric acid-coenzyme A reductase (mevalonate: NADP oxydo reductase (acylating CoA) EC 1.1.1.34) (HMG-CoA reductase). MCNAMARA et al.[9] have shown that rat milk contains a thermostable protein which depresses the activity of HMG-CoA reductase in the liver of adult rats. It seems possible that skim milk from cows contains the protein described by MCNAMARA et al. in rat's milk and that this protein depresses plasma cholesterol levels by partially inhibiting biosynthesis. Further experiments are planned to test this hypothesis.

Summary. From the 15th day of birth, newborn rats were offered rat chow mixed with water or skim milk. The former also received tap water, the latter skim milk. In the latter group, plasma cholesterol was lowered in 43- and 64-day-old males and in 64-day-old females.

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